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Evaluation of *Costus afer* Ker Gawl. Rhizome fractions for hepatoprotective function and characterization of its bioactive compounds

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ABSTRACT

Objective: To investigate the effect of *Costus afer* rhizome fraction on liver function, oxidative stress and inflammation biomarkers in diclofenac-induced hepatotoxicity using rat model. *Method:* Thirty-six male rats of the Wistar strain were separated randomly into six groups, of six each, and treated for 15 days. Diclofenac was administered on day 15. Group I (0.2 mL of 0.9% saline); Group II (200 mg/kg body weight (b.wt.) diclofenac [DF]); Group III (200 mg/kg b.wt. DF + 10 mg/kg b.wt. quercetin); Group IV (200 mg/kg DF + 100 mg/kg b.wt. ethyl acetate fraction [CAERF]); Group V (200 mg/kg b.wt. DF + 300 mg/kg CAERF); and Group VI (200 mg/kg b.wt. DF + 500 mg/kg CAERF). Measurement of oxidative stress, inflammatory biomarkers, plasma albumin, globulin and total protein were performed using spectrophotometric methods. Histology examination was done. CAERF was characterized and analyzed by making use of the gas chromatography-mass spectrometry (GC-MS). *Results:* Findings showed that Groups III-VI had reduced plasma ALT, AST, SOD activities and GSH concentrations, while CAT activity, albumin and globulin levels were elevated when compared with Group II. Furthermore, Groups III-VI had the TNF-α and IL-1β levels significantly reduced ($P<0.05$) when compared with Group II. GC-MS analysis detected 31 compounds. Histological examination showed Group VI had normal histoarchitecture. *Conclusion:* This study showed that CAERF protected against diclofenac-induced hepatic damage in rats. It is recommended that CAERF could be channelled towards pharmaceutical drug development to harness hepatoprotective drug(s).

Keywords: *C. afer*, Diclofenac, Hepatoprotective, Biochemical analysis, Histopathology

1. INTRODUCTION

Hepatotoxicity is the 11th and the 15th leading cause of morbidity and death worldwide, attributed to 1.5% of disability-adjusted life years, and 2.2% of deaths. It has been revealed that a major cause of over 50% acute liver failure is attributed

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to drug-induced liver injury (DILI). The US EPA's Integrated Risk Information System found 134 conventional drugs affecting the liver, including diclofenac (Wahlang et al., 2019). Conventional drugs often cause unwanted side effects, and current synthetic agents often have undesirable side effects (Holland et al., 2022). Discuss how diclofenac induces hepatotoxicity and at what dosage. Physicians and researchers are working to find more effective, safer, and less adverse effects-free alternatives.

Costus afer Ker Gawl has been identified as a medicinal plant with numerous pharmacological activities, including phytochemicals and macro- and micronutrients (Anyasor et al., 2014). It is used in treating inflammatory disorders and liver diseases, with the rhizome extract being used in ethnomedicine to manage liver associated disorders. The plant contains bioactive compounds like polyphenolics, tannins, anthraquinones, terpenoids, alkaloids, and steroidal sapogenins (Nwauche et al., 2018). The aim of this study is to evaluate the hepatoprotective potential of *C. afer* rhizome fraction against diclofenac-induced liver injury in Wistar rats and characterize its chemical constituents of the most active fraction. The study would scientific insight into its mechanistic action in liver disorder management and possibly identify novel compounds for drug production.

2. MATERIALS AND METHODS

Chemicals

All chemicals used were of analytical-reagent grade, and purchased as indicated: Diclofenac sodium from Agram Pharmaceutical Ltd. (Ilishan, Remo, Nigeria); heparin, methanol, ethyl acetate, n-butanol, n-hexane from Avantor Performance Materials B.V (Deventer, Netherlands); Biochemical kits for Alanine aminotransferase, Aspartate aminotransferase, glutathione reduced (GSH), superoxide dismutase (SOD), etc. span from Randox diagnostic kit manufacturer (Randox, United Kingdom).

Preparation of Plant Materials

The rhizome of *C. afer* plant were obtained from Ilara Remo forest, Ilara, Ogun State. Authentication of the plant was carried out at the Forest Herbarium, Ibadan, Oyo State, with authentication number UIH-23244. The rhizomes were washed with water and air-dried in shade. The oven dried material was ground into coarse powder form with the use of mechanical grinder and the powder was stored in tightly sealed glass jars.

Preparation of Extracts

The *C. afer* rhizome powder was extracted using 70% methanol in the ratio of 1:8 (w/v), and extraction process last for 72 hours with intermittent shaking. The methanol extract (70%) was filtered using the Whatman No. 1 filter paper, and the filtrate concentrated subsequently using a rotary evaporator (Stuart 15, Cole-parmer limited, United Kingdom) at a temperature of 40°C. Reconstitution of the concentrate was done with distilled water in the ratio 1:2 and partitioned using the Kupchan et al., (1969) method with modification, of successive solvent portioning done in the following order: hexane; ethyl acetate; n-butanol; and aqueous fractions. The different fractions which include *C. afer* hexane rhizome fraction (CAHRF), *C. afer* ethyl acetate rhizome fraction (CAERF), *C. afer* n-butanol rhizome fraction (CABRF), *C. afer* aqueous rhizome fraction (CAARF), and *C. afer* crude extract rhizome fraction (CACRF) were equally concentrated with the use of rotary evaporator, at 40°C and stored in the refrigerator at 4°C.

Quantitative Determination of Phytochemical Constituents

Determination of total phenolic and total flavonoid contents

The Velioglu et al., (1998) method was used in determining the total phenolic content of *C. afer* rhizome fraction using the Folin-Ciocalteu reagent. The mixture was retained for 5min at room temperature, and 0.75 mL of 6% sodium carbonate was equally added. The absorbence was recorded at 725 nm, after reaction for 90 min. Evaluation of the phenolic concentration was carried out from a gallic acid calibration curve, and determination of the flavonoid content was equally done using the aluminium chloride colorimetric method (Chang et al., 2002). The flavonoid concentration was evaluated from the quercetin calibration curve and the reaction mixture was placed for 30mins at room temperature. The recording of the absorbance of the reaction mixture was done at 415 nm, and the total flavonoids expressed as mg of quercetin equivalent/gram dry weight.

In vitro Antioxidant Activity of *C. afer* Rhizome Fractions

The DPPH radical scavenging activity of *C. afer* rhizome extract/fractions were carried out by using the procedure reported by (McCune and Johns, 2002). Absorbance was determined at 517 nm. The estimation of the capacity of *C. afer* rhizome fractions in scavenging hydrogen peroxide was carried out as described by (Ruch et al., 1989). This assay involves measurement of the

disappearance of H2O2, in the presence of an antioxidant, at a wavelength of 230nm. Calculation of total antioxidant capacity was done according to the method of (Prieto et al., 1999).

In-vitro Anti-inflammatory Activity of the *C. afer* Rhizome Fractions

Preparation of erythrocyte suspension was done according to Okoli method with slight modifications based on (Gunathilake et al., 2018). Studies on the effects of the *Costus afer* rhizome fractions on protein denaturation were done based on the method by Gambhire et al., (2009), with slight modifications as indicated by (Gunathilake et al., 2018).

Characterization of the most active fraction of *C. afer* rhizome by Gas chromatography-mass spectrometry (GC-MS) analysis

The most active fraction was subjected to Gas chromatography-mass spectrometry (GC-MS) analysis for elucidation of the bioactive components. The investigation of CAREF was done on a GC-MS (Thermo Scientific Co.) using the Thermo GC-TRACE ultra version 5.0. Thermo MS DSQ II. Determination of the constituent compounds were carried out by comparison of the retention times and weights of the mass, with that which was gotten from the GC analysis and the mass spectra from National Institute of Standards and Technology (NIST) database and literatures.

Animal Procurement and Care

Thirty-six male albino rats of the Wistar strain, weighing between 120 - 150 g were purchased from Animal Holding Facility, Babcock University, Ogun State, and acclimatized for a period of two weeks using aerated plastic cages at room temperature, using natural light. The rats were fed with rat pellets and water *ad libitum.* The handling of animals was done humanely and maintained as prescribed by the National Institute of Health Animal Care and Use Guidelines (National Institute of Health, 2011). Ethical approval was obtained from the Babcock University Health Research Ethics Committee with the number: BUHREC 713/22.

Animal grouping and dosage regimen

Thirty-six (36) male rats were randomly separated into six groups, consisting of 6 rats per group and properly identified. Administration of the treatment regimen was done orally for 15 days using normal saline as vehicle. Hepatotoxicity was induced using a single dose of 200 mg/kg b.w. diclofenac sodium (DF) on 14th day. Group 1 (Normal: orally administered 0.2 mL of normal saline); Group 2 (Control (untreated): orally administered 200 mg/kg body weight (b.w.) Diclofenac (DF); Group 3 (Standard: administered 200 mg/kg b.w. DF + 10 mg/kg b.w. quercetin); Group 4 (Test I: administered 200 mg/kg DF + 100 mg/kg b.w. most active fraction); Group 5 (Test II: administered 200 mg/kg DF + 300 mg/kg most active fraction); Group 6 (Test III: administered 200 mg/kg DF + 500 mg/kg most active fraction).

Animal sacrifice, sample collection and tissue processing

Blood samples were collected at the end of treatment period, from the retro-orbital venous sinus of rats and were separated for biochemical and inflammatory biomarkers determination. Liver tissues were collected and homogenized in cold Tris aminomethane (TRIS) buffer. The liver and homogenates were cold centrifuged for 15 minutes, and the supernatants were used to evaluate oxidative stress parameters.

Acute Toxicity Profile

The *C. afer* rhizome fraction (ethyl acetate fraction) that exhibited the most anti-inflammatory and antioxidant activities *in vitro,* was subjected to acute toxicity study using animal model. Determination of median lethal dose (LD50) values was then done using the (Up and Down) method in accordance with the organization for economic co-operation and development guideline no. 423.

Biochemical and Inflammatory Biomarkers Analyses

Superoxide dismutase (SOD) activity was measured using Randox diagnostic kit manufacturer (Randox, United Kingdom), based on the protocol of (Magnani et al., 2000). Measurement of CAT activity was done using Randox kit, according to the protocol of the manufacturer. GSH content in the liver homogenate was estimated using Randox kit and the procedure of (Sedlak and Lindsay, 1968). Plasma alanine aminotransferase, total protein, aspartate amino transferase activities, globulin, and albumin were determined according to the procedures as indicated by the manufacturer of the Randox diagnostic kit (Randox, United Kingdom) used. The cytokines (TNF-α and IL-1β) levels in the plasma was evaluated by using the commercial enzyme-linked immunosorbent assay (ELISA) kits as prescribed by the manufacturer.

Histopathological Examination

After anesthetizing, sacrificing, and dissection, liver tissues were excised from the sacrificed animals, and weighed and also fixed in 10% buffered formalin at a neutral form, and processed for embedment in paraffin, after which 5-6 mm sections were cut and made use of eosin and hematoxylin before examining under light microscope, for the histopathological changes (Tousson et al., 2016).

Data Analysis

Data was statistically compared using one-way analysis of variance (ANOVA). The construction of charts and graphs were equally done using GraphPad Prism 9. *p*-value of less than 0.05 ($p<0.05$) were considered to be statistically significant.

3. RESULTS

Quantitative Phytochemical Screening of *C. afer* Rhizome Fractions

Table 1 shows that CAARF (83.33 ± 0.01 GAE mg/g) had a significant ($p<0.05$) high total phenol content while CABRF (58.67 ± 0.01 GAE mg/g) had the least total phenol content. CACRE (4.40 ± 0.01 QE mg/g) had a high flavonoid content while CAERF (2.88 ± 0 .01 QE mg/g) had the least flavonoid content.

Table 1 Quantitative phytochemical screening, TAC and Fifty percent inhibitory concentrations (IC50) of *in vitro* anti-oxidant and anti-inflammatory study fractions *C. afer* rhizome at 20 - 100 µg/ml for DPPH, H2O2 Scavenging activities (Ascorbic acid) and EMS, HPD (Diclofenac sodium)

Test samples	Total Phenol (GAE mg/g)	Total Flavonoid (QE mg/g)	TAC (mg/AAE/g) ± SD	DPPH IC50 (µg/mL)	H2O2 IC50 (µg/mL)	EMS IC50 (µg/mL)	HPD IC50 (µg/mL)
Ascorbic acid	-	-	-	126.25	-	-	-
DF	-	-	-	-	-	33.61	15.33
Crude	75.01 ± 0.01b	4.40 ± 0 .01a	0.134 ± 0.00d	197.24	183.51	64.37	66.21
Butanol	58.67 ± 0.01d	3.01 ± 0 .01d	0.220 ± 0.01a	171.00	216.73	61.48	59.99
Ethyl acetate	70.10 ± 0.01c	2.88 ± 0 .01e	0.142 ± 0.00b	125.26	172.33	60.08	28.44
Aqueous	83.33 ± 0.01a	3.73 ± 0.01b	0.121 ± 0.00c	326.48	197.58	86.82	70.81
Hexane	74.67 ± 0.01b	3.43 ±0 .01c	0.144 ± 0.00b	194.51	210.41	84.56	73.51

GAE- Gallic acid equivalent. QE- Quercetin equivalent. TAC – Total antioxidant Capacity. AAE – ascorbic acid equivalent. DPPH - 1,1 diphenylpycrylhydrazyl and radical scavenging activity. H2O2 - Hydrogen peroxide and radical scavenging activity. DF- Diclofenac sodium. EMS- Erythrocyte membrane stabilization. HPD- Inhibition of protein denaturation. Different letters show indication of values that are significantly different at $p < 0.05$

***In vitro* Antioxidant studies of *C. afer* Rhizome Fractions**

Using ascorbic acid as standard, Table 1 shows that CAERF (IC50 = 125.26 µg/ml) showed a significantly ($p<0.05$) increased DPPH scavenging activity while CAARF (IC50 = 326.48 µg/ml) was the least, compared with other fractions. Table 1 also shows that CAARF (7.62 ± 0.03 mg AAE/g) exhibited a significantly high total antioxidant capacity while CACRE (1.47 ± 0.02 mg AAE/g) was the least, compared to the other fractions. Table 1 also shows that CAERF (172.33 µg/ml) showed a significantly ($p<0.05$) increased H2O2 scavenging activity while CABRF (IC50= 216.73 µg/ml) was the least, compared to the other fractions.

***In vitro* Anti-inflammatory Studies**

Using diclofenac sodium as standard, Table 1 shows that CAERF (IC50 = 60.08 µg/mL) showed a significantly ($p<0.05$) high erythrocyte membrane stabilization activity while CAARF (IC50 = 86.82µg/mL) was the least, compared to the other fractions. Table 1 also shows that CAERF (IC50 = 28.44 µg/mL) showed a significantly ($p<0.05$) enhanced protein denaturation activity while CAARF (IC50 = 73.51 µg/mL) was the least, compared to the other fractions.

Liver Function Tests

Data in Figure 1 illustrates 100 mg/kg b.wt quercetin (12.62 ± 1.31 U/L), 100 mg/kg b.wt *C. afer* (14.58 ± 0.10 U/L), 300mg/kg b.wt *C. afer* (13.32 ± 0.60 U/L) and 500 mg/kg b.wt *C. afer* (12.36 ± 0.55 U/L) treated animals that were induced with toxicity using diclofenac

had significantly (p <0.05) decreased plasma ALT activities when compared with the untreated control (16.01 ± 0.30 U/L). Data in Figure 2 show that 100 mg/kg b.wt quercetin (32.76 ± 1.66 U/L), 100 mg/kg b.wt *C. afer* (45.45 ± 0.91 U/L), 300mg/kg b.wt *C. afer* (40.5 ± 1.97 U/L) and 500mg/kg b.wt *C. afer* (34.66 ± 7.53 U/L) treated animals that were induced with toxicity using diclofenac had significantly (p <0.05) decreased plasma AST activities on comparison with the untreated control (58.98 ± 0.72 U/L).

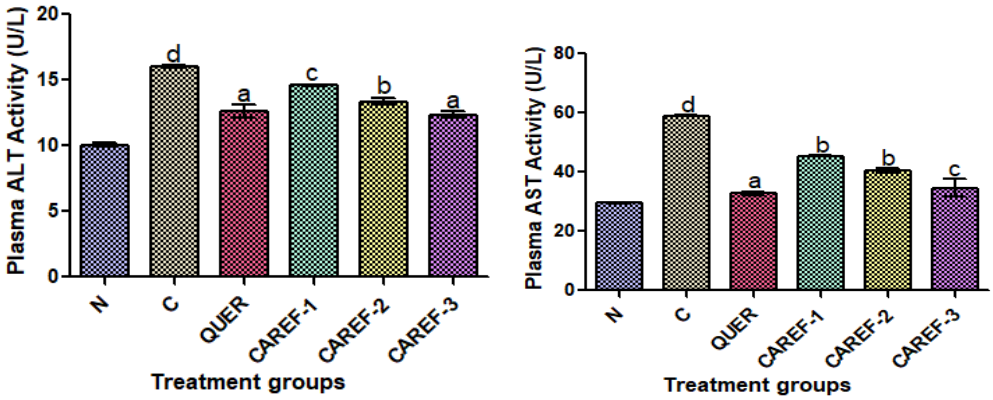

Figure 1 Effects of varying doses of CAERF on plasma alanine aminotransferase (ALT) and plasma aspartate aminotransferase (AST) activity in rats induced with toxicity using diclofenac.
CAERF- *C. afer* ethyl acetate rhizome fraction. N- Normal. C- Control. QUER- Quercetin. CAERF-1- 100mg/kg bwt. CAERF. CAERF-2- 300mg/kg bwt. CAERF. CAERF-3- 500mg/kg bwt. CAERF. Similar letters represent no significant difference between groups. Different letters show values are significantly different at $p < 0.05$

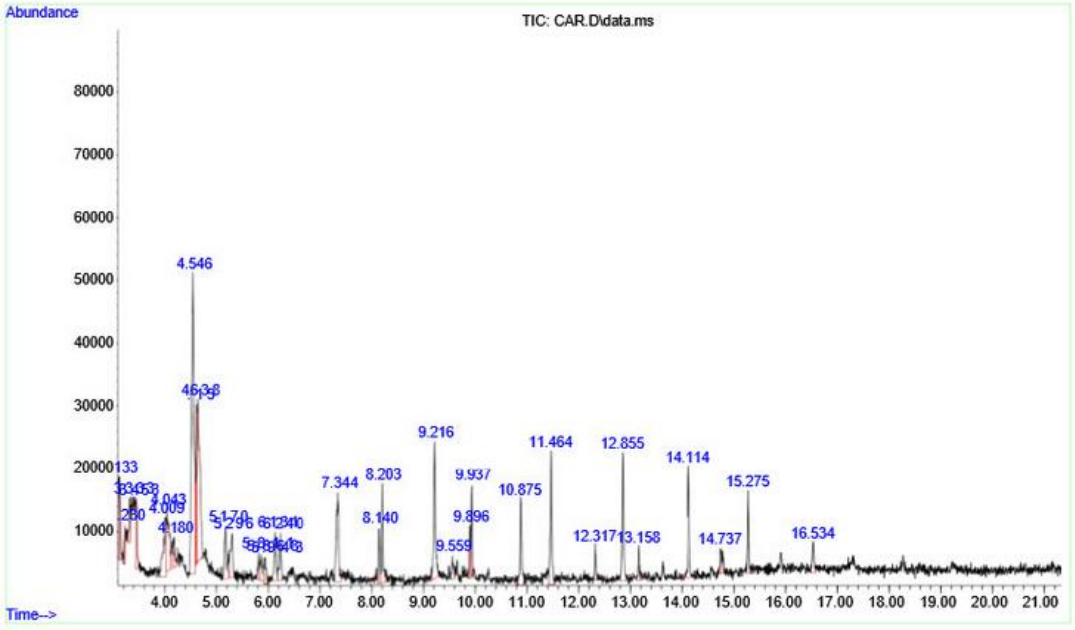

Figure 2 Gas chromatography chromatogram of ethyl acetate fraction of *C. afer* rhizome

In vivo Antioxidant Studies

Table 2 shows that 100 mg/kg b.wt quercetin (1.18 ± 0.24 U/mg protein), 100 mg/kg b.wt *C. afer* (1.26 ± 0.13 U/mg protein), 300 mg/kg b.wt *C. afer* (1.14 ± 0.06 U/mg protein) and 500 mg/kg b.wt *C. afer* (0.83 ± 0.19 U/mg protein) treated animals that were induced with toxicity using diclofenac had significantly (p <0.05) decreased SOD activities when comparing them with the untreated control animals (2.03 ± 0.25 U/mg protein). Table 2 also shows that 100 mg/kg b.wt quercetin (7.01 ± 0.08 U/mg protein), 100 mg/kg b.wt *C. afer* (7.45 ± 0.07 U/mg protein), 300 mg/kg b.wt *C. afer* (9.31 ± 0.03 U/mg protein) and 500 mg/kg b.wt *C. afer* (11.84 ± 0.05 U/mg protein) treated animals induced with toxicity using diclofenac had significantly (p <0.05) elevated CAT activities when

compared with the untreated control animals (3.72 ± 0.02 U/mg protein). Table 2 also shows that 100 mg/kg b.wt *C. afer* (6.71 ± 0.00 U/mg protein), 300 mg/kg b.wt *C. afer* (5.82 ± 0.003 U/mg/protein) and 500 mg/kg b.wt *C. afer* (5.04 ± 0.00 U/mg/protein) treated animals had significantly ($p < 0.05$) decreased GSH level when compared with the untreated control animals (10.82 ± 0.001 U/mg/protein).

Table 2 Effect of CAERF on different biochemical parameters in rats induced with toxicity using diclofenac.

Parameters	Normal	Untreated Control	Standard	100mg/kg b.wt *C.afer*	300mg/kg b.wt *C.afer*	500mg/kg b.wt *C.afer*
SOD	0.63 ± 0.13	2.04 ± 0.24d	1.19 ± 0.24b	1.26 ± 0.13b	1.15 ± 0.05c	0.83 ± 0.19a
CAT	16.72 ± 0.31	3.72 ± 0.02a	7.01 ± 0.08b	7.45 ± 0.07b	9.31 ± 0.03c	11.84 ± 0.05d
GSH	4.64 ± 0.00	10.82 ± 0.00d	7.19 ± 0.01c	6.71 ± 0.01c	5.82 ± 0.00b	5.04 ± 0.00a
Total protein	79.64 ± 0.01	45.71 ± 0.01a	71.68 ± 0.03d	51.93 ± 0.01b	55.91 ± 0.02b	67.38 ± 0.01c
Albumin	23.86 ± 0.09	9.83 ± 0.03a	12.74 ± 0.04b	12.66 ± 0.04b	17.73 ± 0.07c	19.31 ± 0.04d
Globulin	55.78 ± 0.01	35.89 ± 0.01a	58.94 ± 0.01d	39.27 ± 0.01b	38.18 ± 0.01b	48.06 ± 0.01c
TNF-α	10.92 ± 0.00	24.38 ± 0.00e	12.65 ± 0.00a	21.31 ± 0.00d	17.84 ± 0.00c	14.97 ± 0.00b
IL-1β	27.43 ± 0.00	38.64 ± 0.00c	27.95 ± 0.00a	37.09 ± 0.00c	32.26 ± 0.00b	30.36 ± 0.00b

CAERF: *C. afer* ethyl acetate rhizome fraction. SOD: Superoxide dismutase. CAT: Catalase. GSH: reduced glutathione. TNF-α: Tumor necrosis factor alpha. IL-1β: Interleukin -1β. Different letters show values are significantly different across a row at $p < 0.05$. Expressed of values were done as mean ± standard error of mean (SEM), (n = 6).

***In vivo* Anti-inflammatory Studies**

In Table 2, there was a significant decrease ($p < 0.05$) of total protein and albumin concentrations in untreated animals compared to the normal control. There was a significant increase in total protein, albumin and globulin levels ($p < 0.05$) in *C. afer* treated animals compared to the normal control and untreated control. Table 2 also shows that animals treated with 100 mg/kg b.wt *C. afer* (21.31 ± 0.00 ng/mL), 300 mg/kg b.wt *C. afer* (17.84 ± 0.00 ng/mL) and 500 mg/kg b.wt *C. afer* (14.97 ± 0.00 ng/mL) had significantly ($p < 0.05$) reduced TNF-α concentration when compared with the untreated control animals (24.38 ± 0.00 ng/mL). Table 2 also shows that animals treated with 100 mg/kg b.wt *C. afer* (37.09 ± 0.00 ng/mL), 300 mg/kg b.wt *C. afer* (32.26 ± 0.00 ng/mL) and 500 mg/kg b.wt *C. afer* (30.36 ± 0.00 ng/mL) reduced significantly ($p < 0.05$) concentrations of IL-1β when compared with the untreated control animals (38.64 ± 0.01 ng/mL).

Acute Toxicity of *C. afer* Rhizome Ethyl acetate Fractions

Acute oral toxicity study, showed that animals treated with 2000 mg/kg b.wt. and 5000 mg/kg b.wt CAERF showed no observable clinical toxic signs such as depression, lethargy, jaundice, mortality, etc. during and after the observation period of 14 days. Hence, CAERF was considered to be safe, up to the dose 5000 mg/kg b.wt.

GC-MS analysis of ethyl acetate fraction of *C. afer* rhizome

Data in Figure 3 shows the tentative identification of twenty-six compounds in *C. afer* ethyl acetate fraction by Gas Chromatography-Mass Spectrometry analysis. The compound Carbonic acid; decyl prop-1-en-2-yl ester had the highest quantity (16.55%), while Methyl 11-methyl-dodecanoate had the least quantity (0.83%).

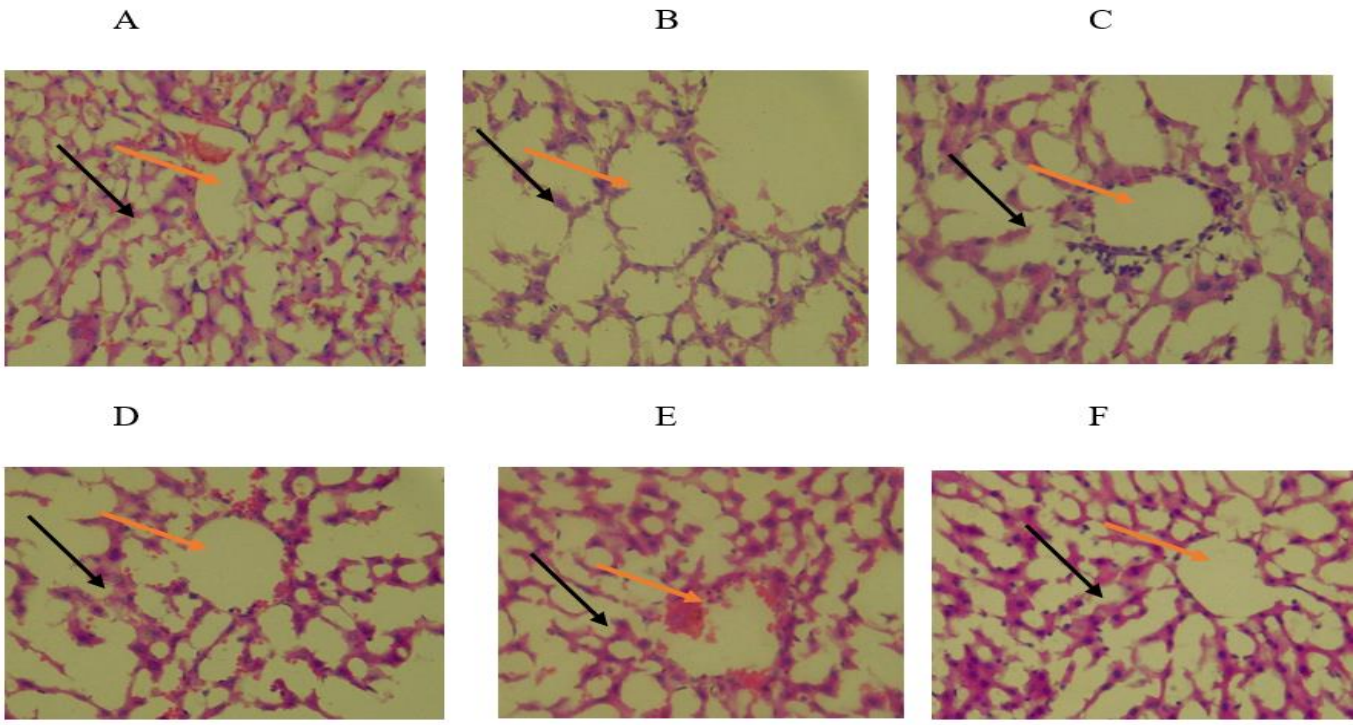

Figure 3 Photomicrograph of the hepatic tissues of test animals. (A) normal group (B) control group (C) standard group (200 mg/kg b.wt DF + 10 mg/kg b.wt. quercetin) (D) 200 mg/kg b.wt DF + 100 mg/kg b.wt. CAERF group (E) 200 mg/kg b.wt DF + 300 mg/kg b.wt. CAERF group (F) 200 mg/kg b.wt DF + 500 mg/kg b.wt. CAERF group.

Histological Examination of the Liver

Normal architecture of the liver lobular alongside radiating hepatic cords and central vein, with intact hepatocytes having sinusoidal spaces were revealed in the histological sections of the normal rat liver slices (Figure 3A). The histopathological examination of the rat liver of the untreated control group showed major liver slices alterations like ballooning, hepatocellular necrosis, degeneration of hepatocytes, and inflammatory cell infiltration (Figure 3B). The standard group (Figure 3C) showed a mild patch of necrotic hepatocytes, and a mild distortion in the central vein, including a lobular inflammation. Further *C. afer* ethyl acetate fraction treatment led to an improvement in morphological features (Figure 3D-F). Therefore, the observed changes in the histological sections indicated substantiated biochemical results.

4. DISCUSSION

This study analyzes the phytochemicals of *C. afer* rhizome, revealing high levels of phenol and flavonoids, indicating its antioxidant activity. Phenolic compounds, including flavonoids, have antioxidant properties against reactive oxygen species (ROS) (Al-Mamary and Moussa, 2021). In addition, the rhizome of *Curcuma caesia* had been reported to contain phenol (Arya et al., 2022). The ethnomedical use of *C. afer* rhizome extract in treating oxidative stress-related disorders may be due to its phytochemicals. The *in vitro* investigation of the anti-inflammatory activity of *C. afer* rhizome fractions revealed that CAERF had a high stabilization effect on hemolysis of human red blood cell membrane induced by heat, suggesting its potential as an anti-inflammatory agent. Drug-induced erythrocyte membrane stabilization is a key indicator of anti-inflammation activity (Jin et al., 2021). CAERF showing a concentration-dependent effect on BSA denaturation.

The ethanoic extract of Curcuma zedoaria displayed an anti-inflammatory effect by inhibiting protein denaturation, according to a similar report by (Ullah et al., 2014). The extract has the potential of being a source for anti-inflammatory compounds. The crude extract and fractions showed significant antioxidant activity, with *in vitro* models showed that *C. afer* rhizome showed a positive indication of a free radical scavenging plant, with CAERF showing the least DPPH scavenging activity. This can be ascribed to the hydroxyl groups being present in the flavonoid and phenolic ring structures of the antioxidant compound (Vuolo et al., 2019). CAERF also demonstrated a significantly high total antioxidant capacity. Reduction of molybdenum to molybdenum may be as a result of the hydrogen and electron-donating potentials of these phenolic compounds present in various test fractions (Khiya

et al., 2021). CAERF's hydrogen peroxide radical scavenging activity indicates its potential antioxidant activity. This led to its selection for animal investigations and GC-MS analysis.

GC-MS analysis of CAERF revealed 26 compounds and these compounds have been reported to exhibit bioactivities such as trans-4-(2-(5-Nitro-2-furyl) vinyl) -2-quinolinamine was reported to exhibit antimutagenic activity Miyazawa and Hisama, (2003), Cyclotetrasiloxane, octamethyl; 2,4-Dihydroxyacetophenone; Carbonic acid, decyl prop-1-en-2-yl ester; Oxalic acid, isobutyl nonyl ester; Tetrasiloxane, 1,1,3,3,5,5,7,7-octamethyl; Silicic acid, diethyl bis(trimethylsilyl) ester; Propenone, 3-(2-benzoxazolylthio)- 1-phenyl-; 2,3,4,5-Tetrafluorobenzyl alcohol, (3-cyanopropyl) dimethylsilyl ethe; were reported to exhibit antimicrobial activity Ejidike and Ajibade, (2015), Arsenous acid, tris(trimethylsilyl) ester; Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-; and 3-Quinolinecarboxylic acid, 6,8-di fluoro-4-hydroxy-, ethyl ester were reported to exhibit antibacterial activity Oloyede et al., (2023), Octasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11,13,13,15,15-hexadecamethyl- was reported to exhibit antifungal activity Jeeva and Krishnamoorthy, (2018), Abdullah et al., (2020), methoxyacetic acid; tetradecyl ester; Heptasiloxane; 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-; Cyclopentasiloxane, decamethyl-; 6,6,8,8,10,10-Hexamethyl-2,5,7,9,11,14-hexaoxa-6,8,10-trisilapentadecane; Hexadecanoic acid; methyl ester; 3-Isopropoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane were reported to exhibit hepatoprotective property Baishya et al., (2018), Kowti et al., (2020), Mohan et al., (2022), 1,2,5-4-(4-methoxyphenoxy)-; Oxadiazol-3-amine; Cyclotetrasiloxane, octamethyl-; 3-Isopropoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane were reported to exhibit antioxidant activity Khurana et al., (2019), Ponnudarai, (2020), Hexahydropyridine, 1-methyl-4-[4,5-dihydroxyphenyl] was shown to increase sex hormone Papach et al., (2022), Cyclohexasiloxane, dodecamethyl-was stated to show antitumor activity Kumar et al., (2018), Nordazepam, TMS derivative was equally reported to show alkylation activity Singh et al., (2012), and Methyl 11-methyl-dodecanoate was observed to exhibit neurological activity Rahman et al., (2021).

These bioactivities could account for C. afer rhizome extracts in ethnomedical practice. CAERF was well-tolerated by test rats, with no visible toxicity or mortality observed. This is an agreement with the previous studies of ethyl acetate fraction of *Cocos nucifera* L. that shows they were no death or signs of toxicity in the rats Ekanayake et al., (2019). Animal studies showed reduced ALT and AST activities in rats administered with CAERF, suggesting hepatoprotective activity. This suggests that CAERF may reduce hepatopathy induced by diclofenac-induced ROS, allowing enzymes to leak into the bloodstream as noted in study done by (Oseni et al., 2018). *In vivo* antioxidant analysis showed that CAERF-treated animals were protected against diclofenac-induced oxidative stressors in a better manner when compared to the untreated control.

Furthermore, the presence of flavonoids and phenols in CAERF may have augmented or complement the endogenous antioxidant defense system, thereby causing the reduction of oxidative stress. This is supported by previous study on *Terminalia berillica* which also shows that animals treated with the medicinal plant were better protected against diclofenac induced oxidative stress (Gupta et al., 2020). Elevated CAT, GSH, and SOD activities are crucial indicators of the body's antioxidant capacity. Furthermore, total protein, globulin, and plasma albumin levels were increased in CAERF treated rats compared to untreated control. This suggests that CAERF could regenerate and enhance the status of the function of the liver after treatment.

When blood proteins (albumin and globulin) are lost excessively during a hepatotoxic condition, the liver's normal functioning is altered, which leads to the buildup of toxic products. Globulin and albumin function as biomarkers and transport proteins in the disease state (Abbas and Humma, 2021). CAERF treatment reduced TNF-α and IL-1β levels in treated groups, indicating anti-inflammatory activity. Overdose of diclofenac induces reactive oxygen species (ROS), which are potent inducers of TNFα and IL-1β cytokines (Niederreiter and Tilg, 2018). CAERF extracts counteract free radical formation, thereby reducing oxidative stress and stopping oxidative damage in the liver.

The histological examination of liver tissues after induction of diclofenac reflects toxicity by producing pathological conditions presented by forming lymphocyte, centrilobular megaloventral vein, severe distortion of the liver parenchymal and neutrophil infiltration. Studies have given a report that diclofenac has the potential to alter the structure of hepatocytes and cause infiltration of inflammatory cells and sinusoidal dilation (Dass and Patel, 2018; Gupta et al., 2020). The result suggests that there was improvement in the morphological features of the liver as a result of CAERF administration. This is consistent with other findings that reported that bioactive compounds present in *C. afer* extract could ameliorate lead induced nephrotoxicity (Ezejiofor et al., 2017; Ezejiofor and Orisakwe, 2019).

5. CONCLUSION

Findings from this present study indicate that CAERF possess the capacity to protect against diclofenac-induced liver injury. It is proposed that the hepatoprotective mechanism of action could be through membrane and protein structure stabilization, antioxidant and anti-inflammatory activities and enhanced tissue injury repair mechanism.

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Informed consent

Not applicable.

Ethical approval

The Animal ethical guidelines are followed in the study for experimentation.

Conflicts of interests

The authors declare that there are no conflicts of interests.

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Data and materials availability

All data associated with this study are present in the paper.

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